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AWARD NUMBER: W81XWH-06-1-299

TITLE: Enhancing the Efficacy of Dendritic Cell Vaccines by Tissue Conditioning

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REPORT DATE: Mar 2009

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 Mar 2009	Final	3 Feb 2006 – 2 Feb 2009

4. TITLE AND SUBTITLE

Enhancing the Efficacy of Dendritic Cell Vaccines by Tissue Conditioning

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0299

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Florida
Gainesville, FL 32611

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
No Abstract was provided.

15. SUBJECT TERMS
No Subject Terms were provided.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	10	USAMRMC

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"Enhancing the Efficacy of Dendritic Cell Vaccines by Tissue Conditioning"

PC 051358 (PI Philipp Dahm)

Progress Report (June 2009)

a. Introduction

The overall objective of this proposal has been to investigate the novel *in situ* immune modulation for enhancing the efficacy of cancer vaccines. The central hypothesis of the approach under investigation is that the *ex vivo* step of DC maturation as a necessary prerequisite for generating migratory DC capable of stimulating strong antigen-specific T cell responses can be replaced by an *in vivo* process termed '*in situ* priming'. Based on preclinical and clinical data from our laboratory and others we hypothesized that the topical immunostimulant Imiquimod is a suitable agent that provides the necessary signals to facilitate DC maturation, migration and effective antigen presentation.[1] Ultimately, the profound immune stimulation by antigen loaded DC would lead to strong tumor-specific immune response.

'In situ priming' approach has many advantages comparing to conventional *in vitro* maturation including (1) that it recapitulates more closely the physiological conditions for DC maturation and hence may lead to a more desirable outcome, namely a more potent immune response, (2) that it eliminates an *in vitro* culture step, which in the setting of this patient-specific cell therapy protocol represents a considerable simplification, and (3) that in situ maturation obviates the dependence on expensive biological reagents used for *ex vivo* DC maturation.

Our preliminary research findings formed the basis of this proposal to investigate the novel concept of in '*in situ* priming' in the setting of a phase I/II clinical study and to perform detailed immunological and molecular studies both of the local injection site and the peripheral blood stream to better understand the mechanisms of DC maturation and T-cell activation by antigen presenting cells.

b. Results

In our study, we translated the novel concept of in situ maturation into a clinical trial setting. In an open label, Phase I/II, safety and toxicity trial we enrolled 15 patients with hormone-refractory or metastatic prostate cancer study subjects to receive six weekly injections of immature, LAMP-hTERT mRNA transfected DC with a constant dose of 1×10^7 administered to a skin site pretreated with topical applications of Imiquimod. Patients in Dose Level 1, 2 and 3, respectively applied a single-use packet (0.25g) of topical Imiquimod (Aldara^{TM}) cream (5%) once, twice or three times prior to DC injection. Skin biopsies were obtained from study subjects before and after topical Imiquimod application to determine molecular and cellular changes at cutaneous DC injection sites. Studies in human skin tissues focused on analyzing Imiquimod-induced changes on the local cytokine environment, activation of TLR-mediated pathways, and potential recruitment of APC into treated skin sites. Furthermore, we analyzed the magnitude, quality, and duration of vaccine-induced $CD8^+$ and $CD4^+$ T-cell responses in the peripheral blood compartment. Peripheral blood samples were collected at frequent, defined time intervals over the treatment course and during the follow-up period. We also analyzed key parameters of the vaccine-induced $CD8^+$ and $CD4^+$ T-cell response by studying its magnitude, quality, and duration in the peripheral blood compartment.

b-1 Specific Aim #1: To conduct an exploratory phase I/II study to test the safety, immunologic, and clinical activity of in situ-activated DC in patients with metastatic PCA.

A total 15 subjects with metastatic prostate adenocarcinoma, stage with either documented metastatic disease (any N+ or M+) or a rising serum PSA despite continued androgen ablation were enrolled and treated in this Institutional Review Board (IRB) and Food and Drug Administration (FDA-) approved study. This phase of the study was completed in its entirety at Duke University Medical Center as reported in the previous progress report from April 2007.

In brief, a total of 3, 6, and 6 study subjects were enrolled and successfully treated at the low, intermediate and high dose, respectively without encountering any therapy-related adverse events. Immunological monitoring revealed the antigen-specific immune responses were successfully induced in 14 of 15 studied subjects. The clinical trial proportion of this application was thereby completed prior to the relocation of the PI to the University of Florida effective September 1st, 2006.

b-2 Specific Aim #2: To assess the local biological response to Imiquimod application by analyzing molecular and cellular changes at cutaneous DC injection sites following topical administration.

To assess the local biological response to Imiquimod application, and specifically, the time and dose-dependent Imiquimod-mediated release of cytokines, we obtained skin biopsies from untreated skin sites as well as biopsies after increasing numbers of Imiquimod applications. As shown in **Figure 1**, we did not observe an increase in tissue levels of most molecules that are known to mature monocyte derived DC, such as IL-4, IL-5, TNF-α, IL-6, IL-1β. Only a small increase in PGE-2 levels was noted at the highest dose level. However, we found significant migratory chemokines such as RANTES and MCP-1 post treatment. These findings suggest that alternative pathways are involved in the in situ maturation process. At the same time, tissue levels of inhibitory molecules such as IL-10 and MIP-1β significantly increased with each application of Imiquimod.

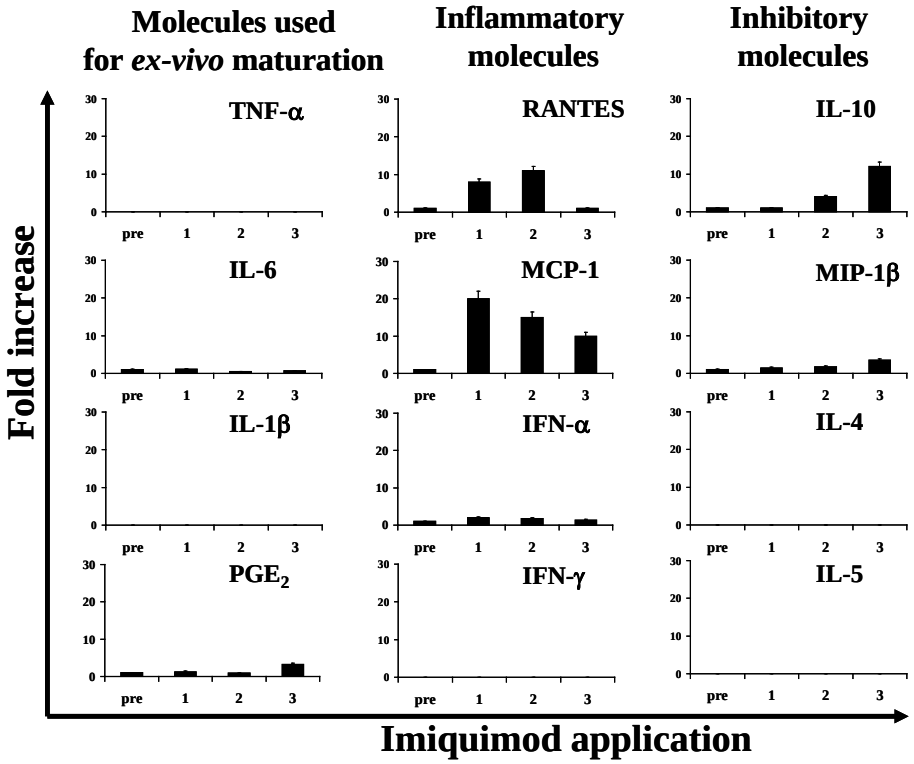

Figure 1. Cytokine profiles in Imiquimod-pretreated dermal tissue. 4 mm skin biopsies were obtained from untreated skin sites (labeled as "pre") as well as skin sites that had undergone a single application (day -1), two applications (days -3 and -1) and three applications of Imiquimod (days -5, -3 and -1). Skin biopsies were obtained at the time of DC injection. Concentrations of RANTES, TNF-α, IL-6, IL-1β, MIP-1β, IL-10, Interferon-α and IL-4 were determined by cytokine bead array according to the manufacturer's instructions. PGE-2 production was quantified by ELISA. Results are presented as cytokine release in Imiquimod-treated tissue vs. untreated control tissue as fold increase Representative results from 3 subjects in each group are shown.

Next, we explored the possibility of indirect maturation of monocyte-derived DC by Imiquimod. As shown in **Figure 2**, we found a novel maturation mechanism of DC induced by the cytokines released from the co-culture of Langerhans cells with Immiquimod. Although Immiquimod alone did not have significant impact on DC maturation, the cytokines released from the activation of Langerhans cells by Immiquimod served as profound stimulation reagents for DC. Thus as a result, immature DC were activated under the skin and migrated to the draining lymph nodes.

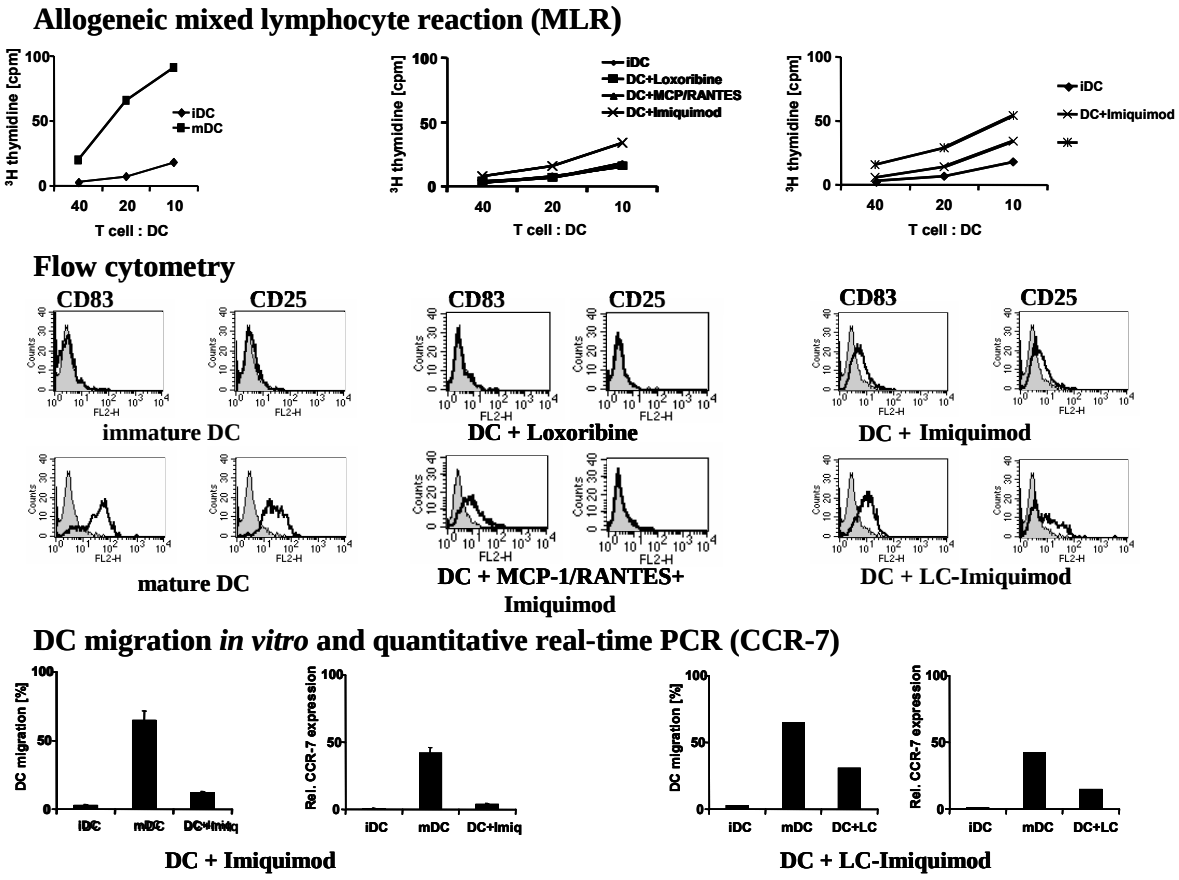

Figure 2. Indirect maturation of monocyte-derived DC by Imiquimod. Immature DCs were activated with different reagents including: maturation cocktail; Loxoribine; Immiquimod and supernatant from LC treated with Imiquimod. The antigen stimulation function of activated DC were tested in the standard MLR reaction (**Upper panel**) and the phenotypes of DC were monitored with FACS analysis (**Middle Panel**). To study the migratory properties of activated immature DC, we performed in vitro migration assays using migratory chemokines and the quantitative real-time PCR using CCR-7 expression as the indicator for DC migration shown in the **lower panel**.

b-3 Specific Aim #3: To analyze the magnitude, quality, and duration of vaccine-induced CD8$^+$ and CD4$^+$ T-cell responses in the peripheral blood compartment.

To analyze the magnitude, quality, and duration of vaccine-induced CD8$^+$ and CD4$^+$ T-cell responses in the peripheral blood compartment we collected peripheral blood samples at defined time intervals over the treatment course and during the follow-up period. Frequencies of hTERT specific T cells before, during and after immunization were measured by IFN-γ ELISPOT. In addition to antigen-specific CD8 T-cell response, we also focused on analyzing the suppressive T-cell responses using the blood samples collected from the low dose and intermediate dose group. Interestingly, we did not observe any activation of FoxP3 positive regulatory T cell and IL-5 secreting Th2 T cells, a finding which is commonly associated with in vitro matured DC. Vaccine. Vaccination with immature hTERT RNA transfected DC resulted in the consistent stimulation of robust hTERT-specific CD8+ T cell responses that appeared of greater magnitude in the patients in the intermediate dose group.

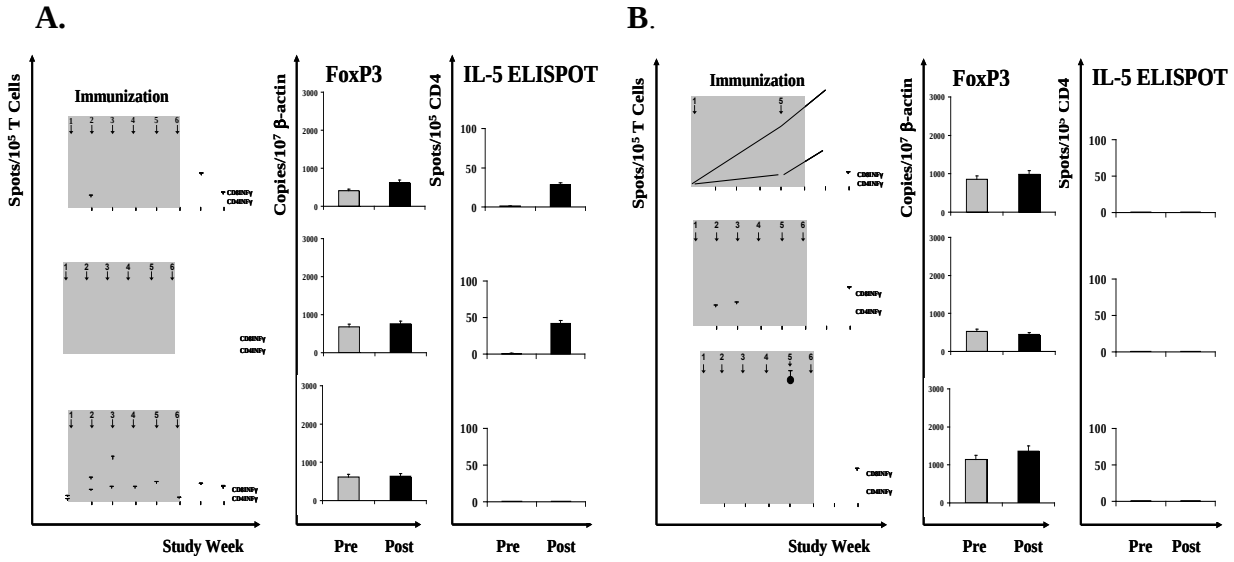

Figure 3. Temporal evolution of hTERT-specific CD8$^+$ T and CD4$^+$ T cell responses in the low dose (**A**) and medium dose groups (**B**). **Left Panel**. Representation of longitudinal CD8$^+$ T and CD4$^+$ T cell responses of three individual patients treated in each treatment arm. **Middle Panel**. FoxP3 RT-PCR analysis on pre and post vaccine CD4 T cells from three patients treated in the intermediate group. **Right Panel.** CD4 T cells were sorted from pre and post vaccination samples and IL-5 ELISPOT analysis were performed to determine the antigen-specific Th-2 T-cell response.

The T cell response from high dose group was also measured with standard ELISPOT analysis and quantitative real-time PCR analysis. The anti tumor T-cell response appeared dampened by the rising number of suppressive T cells. In contrast a continuous increase in the number of antigen-specific T cells observed in the low and medium dose, the frequency of interferon-γ secreting T cells were reduced after the third vaccination. The reduction of antigen-specific T cells was concurrent with an increase of FoxP3 copies in the CD4 T cell compartment which suggests an activation of antigen-specific regulatory T cells. Furthermore, the cytokine profile of high dose treated skin site which comprised high level of IL-10 may preferentially facilitate the induction of Th-2 lineage and suppressive T cell lineage (**Figure 4**).

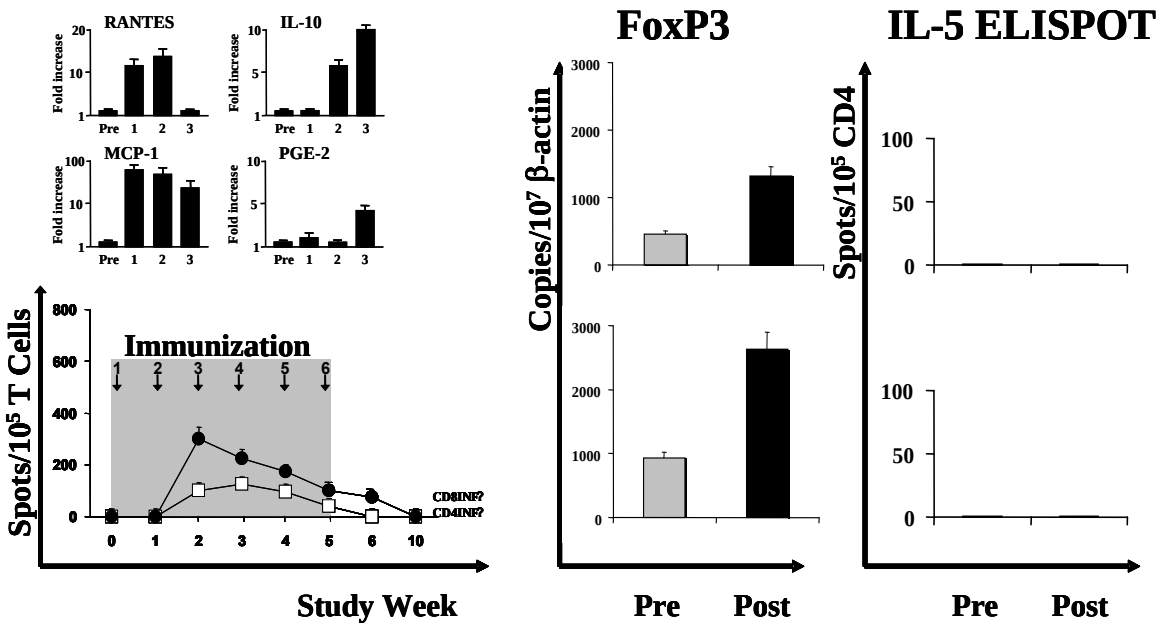

Figure 4. Immune monitoring for high dose group. hTERT-specific $CD8^+$ T and $CD4^+$ T cell responses of a representative patient in the high dose group. **Upper Left Panel.** Cytokine profile in high dose Immiquimod treated dermal tissues. **Lower Left Panel**. Representation of longitudinal $CD8^+$ T and $CD4^+$ T cell responses of three individual patients treated in each treatment arm. **Middle Panel**. FoxP3 RT-PCR analysis on pre and post vaccine CD4 T cells from three patients treated in the intermediate group. **Right Panel.** CD4 T cells were sorted from pre and post vaccination samples and IL-5 ELISPOT analysis were performed to determine the antigen-specific Th-2 T-cell response.

Finally, to access the clinical response after the treatment, serum PSA levels were measured before and after therapy and PSA doubling times were calculated. A total of 15 patients with a follow-up of at least 2 months were available for analysis. Although no objective clinical responses were noted after vaccination, PSAdt calculations revealed a prolonged PSAdt in all three dosing groups (**Figure 5**).

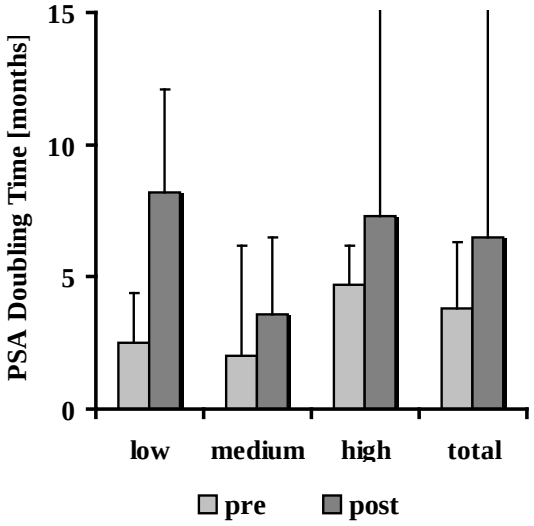

Figure 5. Clinical response to vaccination
Serum PSA was measured before and after therapy and PSAdt was calculated in all three dosing groups.

b-4 Training and research integration

The PI successfully completed all requirements of the Duke Clinical Research Training Program (CRTP) and graduated from this program with a Masters degree of Health Sciences in Clinical Research in June of 6/2006. Although not specifically related to this specific proposal, yet undoubtedly as the result of the opportunities it has afforded him, the PI has developed considerable expertise in research methodology and become one of the leading proponents of evidence based medicine in urology. Several peer-reviewed publications document this positive career development.[2-11] He has recently directed an 1 ½ day workshop on evidence based medicine at the headquarters of the American Urological Association and is one of few surgeons to be invited as faculty of the prestigious workshops on how to teach evidence based medicine at Duke, McMaster and Oxford University. He continues to be intimately involved with translational research in the cancer immunotherapy serving as the Co-PI of two Phase I/IIa studies of RNActive®-derived therapeutic vaccine and active immunotherapy using telomerase mRNA transfected DC applied in a prime boost format, both in patients with metastatic prostate cancer.

c. Key research accomplishments

a) Successful translation of the "in situ maturation" concept into the clinic by executing an innovative phase I/II study and treating patients with advanced prostate cancer with hTERT RNA transfected DC.

b) Our results confirm that the in situ activation of DC with Imiquimod is safe and feasible and leads to the induction of hTERT-specific immune responses.

c) Analysis of the cytokine profiles of Imiquimod treated DC skin injection sites suggest that alternative pathways are involved in DC maturation in the in situ maturation approach.

d. Conclusions

The findings of this study will provide the basis for subsequent clinical trials seeking to establish the therapeutic efficacy of this vaccine platform which are currently in development. A publication in the peer-reviewed literature is in preparation. Meanwhile the PI has co-authored several publications in related topics in the interval.[12, 13] Finally, the protected research time which this Physician Research Training Award has afforded him has permitted him to successfully pursue a Masters of Health Sciences in Clinical Research which has been tremendously valuable to his career development.

e. References

1. Nair, S., McLaughlin, C., Weizer, A., Su, Z., Boczkowski, D., Dannull, J. et al.: Injection of immature dendritic cells into adjuvant-treated skin obviates the need for ex vivo maturation. J Immunol, **171:** 6275, 2003

2. Dahm, P., Preminger, G. M.: A Users' Guide to the Urological Literature: Introducing a Series of Evidence Based Medicine Review Articles. Journal of Urology, **178:** 1149, 2007

3. Scales, C. D., Jr., Norris, R. D., Keitz, S. A., Peterson, B. L., Preminger, G. M., Vieweg,

J. et al.: A critical assessment of the quality of reporting of randomized, controlled trials in the urology literature. J Urol, **177:** 1090, 2007

4. Scales, C. D., Jr., Preminger, G. M., Keitz, S. A., Dahm, P.: Evidence Based Clinical Practice: A Primer for Urologists. J Urol, 2007

5. Bajammal, S., Dahm, P., Scarpero, H. M., Orovan, W., Bhandari, M.: How to use an article about therapy. J Urol, **180:** 1904, 2008

6. Scales, C. D., Jr., Dahm, P.: The critical use of population-based medical databases for prostate cancer research. Curr Opin Urol, **18:** 320, 2008

7. Scales, C. D., Jr., Norris, R. D., Preminger, G. M., Vieweg, J., Peterson, B. L., Dahm, P.: Evaluating the evidence: statistical methods in randomized controlled trials in the urological literature. J Urol, **180:** 1463, 2008

8. Tseng, T., Dahm, P., Poolman, R. W., Preminger, G. M., Canales, B. J., Montori, V. M.: Users' Guide to the Urological Literature: How to Use a Systematic Literature Review and Meta-Analysis. Journal of Urology, **180:** 1249, 2008

9. Tseng, T. Y., Breau, R. H., Fesperman, S. F., Vieweg, J., Dahm, P.: Evaluating the evidence: the methodological and reporting quality of comparative observational studies of surgical interventions in urological publications. BJU Int, 2008

10. Tseng, T. Y., Dahm, P., Poolman, R. W., Preminger, G. M., Canales, B. J., Montori, V. M.: How to use a systematic literature review and meta-analysis. J Urol, **180:** 1249, 2008

11. Yeung, L. L., Fesperman, S. F., Algood, C. B., Rosser, C. J., Vieweg, J., Dahm, P.: Clinical Practice Guidelines for Prostate Cancer: A Critical Appraisal of Methogical Quality. Presented at the American Urological Association Annual Meeting, Orlando, FL, 2008

f. Publications

12. Vieweg, J., Su, Z., Dahm, P., Kusmartsev, S.: Reversal of tumor-mediated immunosuppression. Clin Cancer Res, **13:** 727s, 2007

13. Kusmartsev, S., Eruslanov, E., Kubler, H., Tseng, T., Sakai, Y., Su, Z. et al.: Oxidative Stress Regulates Expression of VEGFR1 in Myeloid Cells: Link to Tumor-Induced Immune Suppression in Renal Cell Carcinoma. J Immunol, **181:** 346, 2008